

Chemically_competent_E._coli

Date: 2024-05-15

Category: Protocols

Created by: Robin Schüren

Before starting

- 1 Required:
Ice, sterile 1.5 mL tubes (label before starting), pre-cooled centrifuge
 - 100 mM CaCl₂, glycerol, both sterile
 - 85 mM CaCl₂ with 15% glycerol

!!! Always work under sterile conditions to avoid contamination !!!

Inoculation


- 2 Inoculate a 5 ml overnight-culture in LB-medium without antibiotics (for DH5a) or with appropriate antibiotics (for other strains).
Grow over night at 37 °C at 250 rpm.

 Luria-Bertani (LB) broth, makes 1L **Amresco Catalog #K488**


Dilution

- 3 Inoculate 100 ml LB-medium with 1/100 volume of the overnight culture and incubate to an OD₆₀₀ = 0.5 - 0.6 at 37 °C and 230 rpm.
- 4 Transfer cells to two sterile 50 ml falcon tubes and incubate on ice for 20 min.

From this step on, always keep cells cold.


 00:20:00

- 5 Centrifuge for 5 min at 4500 rpm and 4 °C (pre-cool centrifuge) and discard the supernatant.


 00:05:00

CaCl₂

- 6 Carefully suspend each pellet in 20 ml of ice-cold 100 mM CaCl₂ and incubate on ice for 1 h.

 01:00:00

- 7 Centrifuge for 5 min at 4500 rpm and 4 °C (pre-cool centrifuge) and discard the supernatant.

 00:05:00

CaCl₂ + glycerol

- 8 **Carefully** suspend each pellet in 2 ml icecold 85 mM CaCl₂ containing 15% Glycerol.

www.protocols.io/print/preparation-of-chemically-competent-cells-n8mdhu6

15:35

Protocol printing

Aliquots

- 9 Pipette 50 - 200 µl aliquots in prepared, pre-cooled 1.5 ml tubes (work on ice), shock-freeze in liquid nitrogen or on dry ice and store at -80 °C.

For DNA transformation, thaw competent cells on ice.

Attached files

image.png

sha256: 2d8734fd562bffc25d3375919f43d791163a0e405b4130466bbf104193ffadb7



image.png

sha256: 2d8734fd562bffc25d3375919f43d791163a0e405b4130466bbf104193ffadb7



image.png

sha256: 95627f8914ac12e386287bab050d24cf1a057573545907e2ac35986a96d2321e



Protocol-Competent-E.-coli.pdf

sha256: 0bc592278a686e24c76e23fb71bd31d06dcf388c50ad4667f6bc5f0a4b221988



Unique eLabID: 20240515-5eb9c3231b0f6a6c0a0cb154804f2f2558a6fd9e
Link: <https://elabftw-lehre.hhu.de/database.php?mode=view&id=157>

Apr 05, 2018

Preparation of chemically competent cells

Forked from [Preparation of chemically competent E. coli cells](#)

DOI

dx.doi.org/10.17504/protocols.io.n8mdhu6Anna Behle¹¹Institute for Synthetic Microbiology

Axmann Lab

iGEM Duesseldorf 2018



Anna Behle

Institute for Synthetic Microbiology

OPEN  ACCESSDOI: dx.doi.org/10.17504/protocols.io.n8mdhu6**Protocol Citation:** Anna Behle 2018. Preparation of chemically competent cells. **protocols.io**<https://dx.doi.org/10.17504/protocols.io.n8mdhu6>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited


Protocol status: Working**We use this protocol and it's working****Created:** April 04, 2018**Last Modified:** April 05, 2018**Protocol Integer ID:** 11245


Abstract

Standard protocol to prepare cells for highly efficient DNA uptake using CaCl₂.


Materials

STEP MATERIALS

 Luria-Bertani (LB) broth, makes 1L **Amresco Catalog #K488**

 Luria-Bertani (LB) broth, makes 1L **Amresco Catalog #K488**

Protocol materials

 Luria-Bertani (LB) broth, makes 1L **Amresco Catalog #K488**

In Materials, Materials, Step 2


Before starting

- 1 Required:
Ice, sterile 1.5 mL tubes (label before starting), pre-cooled centrifuge
 - 100 mM CaCl₂, glycerol, both sterile
 - 85 mM CaCl₂ with 15% glycerol

!!! Always work under sterile conditions to avoid contamination !!!

Inoculation


- 2 Inoculate a 5 ml overnight-culture in LB-medium without antibiotics (for DH5a) or with appropriate antibiotics (for other strains).
Grow over night at 37 °C at 250 rpm.

 Luria-Bertani (LB) broth, makes 1L **Amresco Catalog #K488**


Dilution

- 3 Inoculate 100 ml LB-medium with 1/100 volume of the overnight culture and incubate to an OD₆₀₀ = 0.5 - 0.6 at 37 °C and 230 rpm.
- 4 Transfer cells to two sterile 50 ml falcon tubes and incubate on ice for 20 min.

From this step on, always keep cells cold.


 00:20:00

- 5 Centrifuge for 5 min at 4500 rpm and 4 °C (pre-cool centrifuge) and discard the supernatant.


 00:05:00

CaCl₂

- 6 Carefully suspend each pellet in 20 ml of ice-cold 100 mM CaCl₂ and incubate on ice for 1 h.

 01:00:00

- 7 Centrifuge for 5 min at 4500 rpm and 4 °C (pre-cool centrifuge) and discard the supernatant.

 00:05:00

CaCl₂ + glycerol

- 8 **Carefully** suspend each pellet in 2 ml icecold 85 mM CaCl₂ containing 15% Glycerol.

Aliquots

- 9 Pipette 50 - 200 μ l aliquots in prepared, pre-cooled 1.5 ml tubes (work on ice), shock-freeze in liquid nitrogen or on dry ice and store at -80 °C.

For DNA transformation, thaw competent cells on ice.



SOC Outgrowth Medium protocol by NEB and Qiagen

Date: 2024-05-13

Tags: media E. coli competent cells

Category: Protocols

Status: Verified

Created by: Said Laibacher

1X SOC Outgrowth Medium:

2% Vegetable Peptone or 2% Tryptone

0.5% Yeast Extract

10 mM NaCl

2.5 mM KCl

10 mM MgCl₂

10 mM MgSO₄

20 mM Glucose

For 500ml:

10.000 g Peptone or Tryptone

2.500 g Yeast extract

0.292 g NaCl

0,093 g KCl

0,476 g MgCl₂

0.602 g MgSO₄

1,802 g Glucose

Note: add Glucose after autoclaving the solution with the remaining ingredients, and letting it cool down. Sterilize the final solution by passing it through a 0.2 µm filter!

Use MilliQ Water to reach final volume.



Unique eLabID: 20240513-b0990252bdc21851bb2fbf1da95b1d27a96a708f

Link: <https://elabftw-lehre.hhu.de/database.php?mode=view&id=155>

Electroporation transformation of *K. xylinus*

Date: 2024-05-13

Tags: K. xylinus

Category: Protocols

Created by: Meggie Weber

Title: Transformation of *K. rhaeticus/xylinus*

Our aim: Several Knockouts

We want to get DNA into *K. rhaeticus/xylinus*. Therefore we need a electroporation transformation, so that the DNA can get through the cell wall and into the genom of *K. rhaeticus/xylinus*.

Materials:

Plasmid DNA	
Electrocompetent <i>K. rhaeticus</i> cells (in 50 µl or 100 µl aliquots)	in 50 µl or 100 µl aliquots
Ice bucket and ice	
1 mm path electrocuvettes	
1.5 mL microcentrifuge tubes or PCR tubes	1.5 ml
An electroporator - set at 2.5 kV and 5.9 ms	
HS+cellulase medium	
HS agar plates with appropriate antibiotic	
One aliquot of electrocompetent cells for negative and one for positive control.	

Methods:

1. Set up the electroporator with settings at **2.5 kV**, **5-8 ms**, **400 Ohm** resistance, **25 µF** capacitance.
2. Prepare **15 mL culture** tubes containing **800 µL HS+cellulase** media.
3. Prepare ice bucket, place plasmid DNA and electrocuvettes on ice and thaw electrocompetent cells on ice. NB! Make sure DNA is desalinated before use – ionic solutions can cause arcing.
4. Add **2 µL (20-200 ng/µL) of plasmid DNA** to **100 µL of concentrated cells** in a cold microcentrifuge

or PCR tube and mix well by pipetting. Don't add plasmid DNA to one aliquot of electrocompetent cells for negative control.

5. Transfer the cell/DNA mixture to a cold electroporation cuvette. Dry any water condensate outside of the cuvette, place the cuvette into the electroporator, and apply the pulse.

6. Transfer the pulsed cells into **800 µL of HS+cellulase** medium in a culture tube.

7. Incubate the culture tubes with shaking (170 rpm) at 30 °C for **4 hours - 16 hours** (large incubation time does not result in reduced efficiencies).

8. When culturing for **4 hours**, spin the culture at max available **RPM for 10 minutes**, resuspend in **200 µl and plate** on an HS-agar plate with an appropriate antibiotic concentration.

1. NB! Required antibiotic concentrations are higher for K. rhaeticus than for E. coli. For plasmids pSEVA321, pSEVA331 and pSEVA351 the appropriate chloramphenicol concentration is 340 µg/mL and for pBla-VHb-122 and pBAV1k, the kanamycin concentration is 500 µg/mL. When other backbones are used, antibiotic concentrations may need to be re-evaluated to be suitable for the plasmid.

9. Grow plates at 30 °C inverted, colonies will appear in **24-72 hours**

References:

Gilbert, C., Tang, TC., Ott, W. *et al.* Living materials with programmable functionalities grown from engineered microbial co-cultures. *Nat. Mater.***20**, 691–700 (2021).

<https://doi.org/10.1038/s41563-020-00857-5>

-->

<https://www.pnas.org/action/downloadSupplement?doi=10.1073%2Fpnas.1522985113&file=pnas.1522985113.sapp.pdf>

Attached file

pnas.1522985113.sapp.pdf

sha256: e1e4e85781cb70326f73bbb275ac73ddfdee83e0c72a86ba104f75bce83e3a8b

